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Providing a reaction tube that contains a first reaction mixture having a first primer and deoxynucleoside triphosphates in a top portion of the tube, a second reaction mixture having a second primer and a DNA polymerase in a lower portion of the tube, and a layer of wax separating the first reaction mix from the second reaction mixture

<u>102</u>

Adding a biological sample to the first reaction mixture and incubating the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer

104

Elongating the extension product

Admixing the extension product with the second reaction mixture by melting the wax layer

108

106

Amplifying the extension product by real-time PCR

<u>110</u>

Quantifying the amplified extension product using a control template

112

100

Fig. 1

XXTTAGGGXXXXXXXX (1st primer)

1

Telomerase-mediated

XXTTAGGGXXXXXXXXXXX(TTAGGG),TTAGGGTTAGGG

(extension product)

XXTTAGGGXXXXXXXX (unextended 1st primer)

1

Template-independent elongation

XXTTAGGGXXXXXXXXX(TTAGGG),TTAGGGTTAGGGAAAAAAAAAA(A)_m (elongated ext. product)
AATCCCAATCCCTTTTTTTTT (2nd primer)

. XXTTAGGGXXXXXXXAAAAAAAAAAA(A)_m (elongated 1st primer)
TTTTTTTTT (2nd primer)

AATCCCAATCC

Fig. 2

300

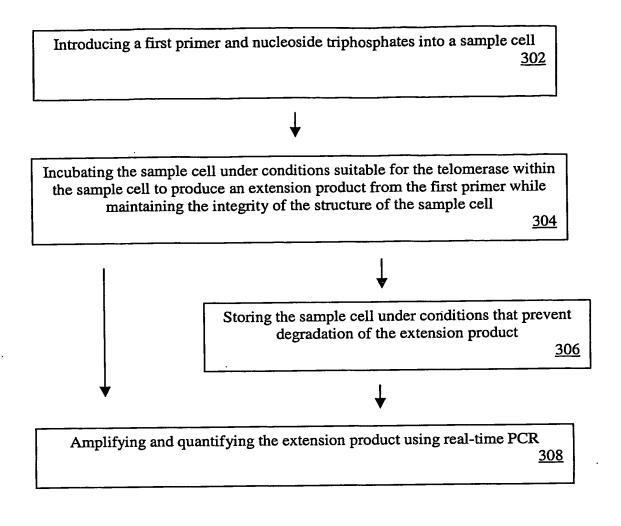
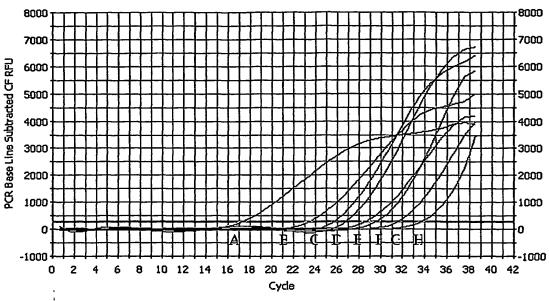


Fig. 3

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- A. 8,500 cells, B. 2,500 cells,
- C. 500 cells, D. 100 cells

- E. 20 cells,
- F. 4 cells,
- G. 1 cell,
- H. Blank.

Fig. 4A

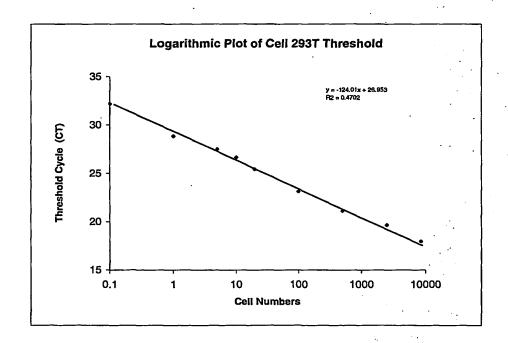
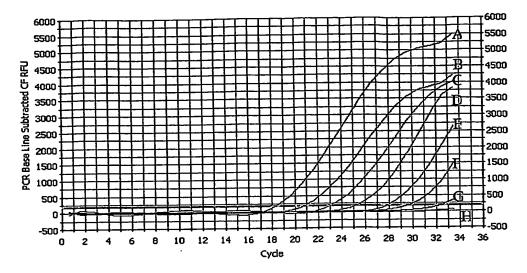


Fig. 4B

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- A. 300,000 molecules, B. 60,000 molecules,
- C. 12,000 molecules,

- D. 2,400 molecules,
- E. 480 molecules,
- F. 100 molecules,

- G. 20 molecules,
- H. Blank.

Fig. 5A

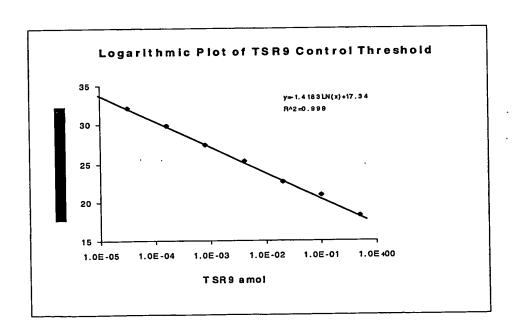
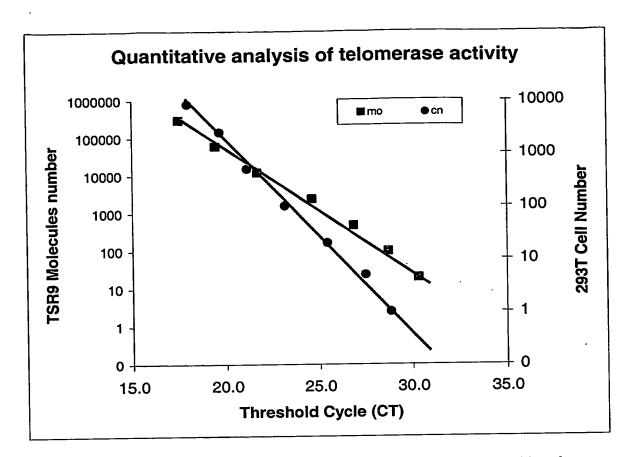


Fig. 5B



mo: Linear regression between TSR9 molecule number and threshold cycle,

cn: Linear regression between 293T cell number and threshold cycle.

Fig. 6

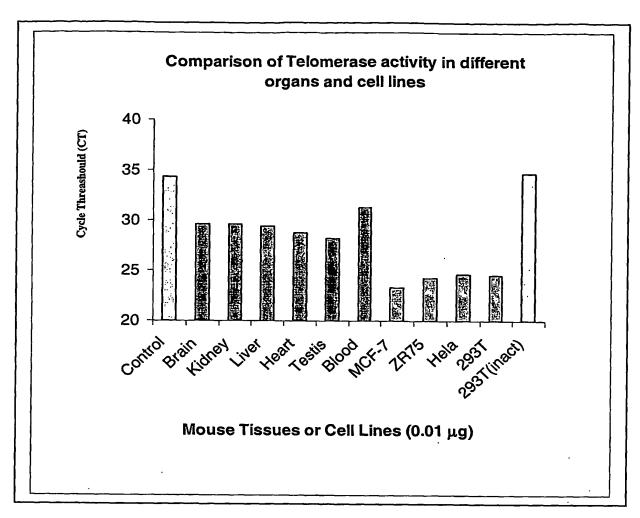
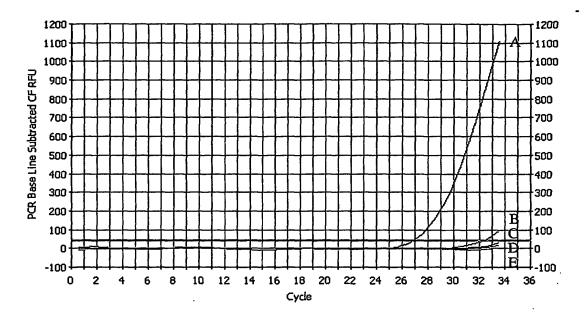


Fig. 7

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A: syringe-treated cells incubated with TS prime;

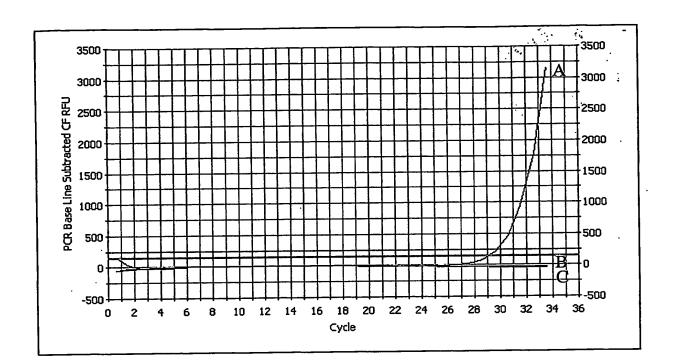
C: untreated cells incubated with TS primer

E: blank.

B: syringe-treated cells incubated without TS primer;

D: untreated cells incubated without TS primer;

Fig. 8



A; with TS primer, B: without TS primer, C: blank

Fig. 9